

Article Addendum

Improving rhizome yield and quality of *Paris polyphylla* through gibberellic acid-induced retardation of senescence of aerial parts

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Abbreviations: GA, gibberellin; GA₃, gibberellin A₃; ABA, abscisic acid; AB, apical bud of rhizome; AP, annual part of rhizome; OP, other parts of rhizome

Key words: rhizome yield, rhizome quality, gibberellin, *Paris polyphylla*, herbaceous perennial, senescence

Senescence in perennials has not been extensively studied compared to that in annual plants, and the effects of delaying senescence on plant biomass and metabolic features in herbaceous perennials has also been poorly reported. We recently examined the effects of gibberellin A₃ (GA₃) on senescence of aerial parts of *Paris polyphylla*, which characterize the metabolic changes associated with senescence, and found antagonistic effects of GA and abscisic acid during this process. Rhizome yield and quality (saponin content) of *Paris polyphylla* were both improved by GA₃-induced retardation of senescence. We propose that GA₃-induced retardation of senescence increases green leaf area and prolongs the duration of photosynthesis, leading to increased rhizome yield. The increased saponin accumulation in GA₃-treated plants may be explained by the longer growth phase and ensuing increased environmental stress.

Senescence is a highly regulated and genetically controlled process that leads to death of plant parts or whole plants.^{1,2} The induction of a series of metabolic changes during this process allows remobilization and recycling of nutrients and helps plants to resume growth after adverse climatic conditions.^{2,3} Senescence is subject to regulation by internal factors, such as hormonal status and reproductive development, and by environmental factors, such as extreme temperature, drought, waterlogging, photoperiod, mineral deficiency and pathogenic infection, through their effects on the internal factors.² Senescence may limit the yield of crop

plants, affect post-harvest quality, or lead to loss of vegetative parts; therefore, improved understanding of senescence may provide a means to prevent these processes and improve crop traits.^{3,4}

Most of the established knowledge on senescence is based on studies of annual plants, and senescence in perennials has not been extensively examined.² Although the cellular, biochemical and transcriptional events during senescence are similar in annual and perennial plants, the effects of delaying senescence on plant biomass and metabolic features of the process in perennial plants, e.g., rhizomatous species, have been less studied. *Paris polyphylla* is a herbaceous perennial in the Trilliaceae, whose rhizomes are used in traditional Chinese medicine as a hemostatic, antimicrobial and anti-inflammatory agent.⁵ This plant takes 7–10 years from seed planting to mature root harvesting, which is too long to meet the demands of the pharmaceutical industry; therefore, studies on how to improve the yield are necessary.

Gibberellin (GA) treatment is known to delay senescence in several species.^{6–11} Thus, to further understand senescence in herbaceous perennials and to investigate whether GA antagonizes abscisic acid (ABA) during the senescence process, we recently examined the effects of gibberellin A₃ (GA₃) on the senescence of aerial parts of *P. polyphylla*.¹¹ GA₃ treatment retarded senescence of the aerial parts and thus increased their longevity. GA and ABA played antagonistic roles during the senescence process, indicating a mechanism of cross-talk among hormones. The senescence process is associated with oxidative stress and is regulated not only by endogenous hormones (e.g., GA, ABA), but also by extrinsic factors (e.g., adverse climate conditions). We also proposed a model for the effects of exogenous GA₃ treatment on the senescence of aerial parts of *P. polyphylla*.

The results of this experiment may be useful in improving the yield and shortening the culture period of *P. polyphylla* by extending the growth phase. GA₃-induced retardation of senescence increased the green leaf area compared to the control and prolonged the duration of photosynthesis. We measured rhizome yield after the aerial parts of *P. polyphylla* had died back completely, and found that it was 7.6% higher after GA treatment (Fig. 1).

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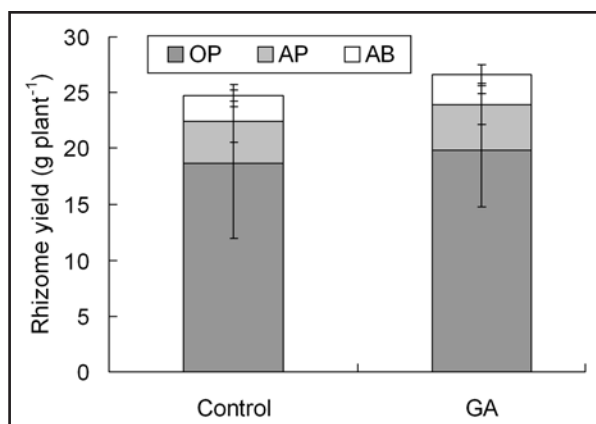


Figure 1. Rhizome yield of apical buds (AB), annual parts (AP) and other parts (OP) in 100 mg L⁻¹ GA₃-treated and control *P. polyphylla*. The rhizomes of the GA₃-treated and control plants were excavated when the aerial parts of the plants completely senesced. AB, AP and OP were isolated and used to measure fresh mass. Values shown represent means (\pm SE) of 30 plants per treatment.

Yields of apical buds (AB), annual parts (AP) and other parts (OP) of rhizome increased by 14.3%, 7.7% and 6.7%, respectively, due to the delayed senescence. Senescence is thought to be an evolutionarily selected developmental processes in which plant cells undergo highly ordered disassembly through cellular metabolism and degeneration of cellular structures, leading to nutrient export to younger, reproductive or storage organs.^{3,4} For *P. polyphylla*, nutrients are remobilized from aerial parts (source) to rhizomes (sink) during senescence. Our results are in agreement with other reports that delayed senescence increases plant biomass or grain yield.¹² Dry matter and yield are improved by prolonging leaf longevity.¹³ Late-senescing lines of *Arabidopsis* accumulate more dry matter in rosette leaves than early-senescing lines.¹⁴ The “stay-green” hybrid of maize has higher grain yield than other hybrids.¹⁵ Moreover, our data suggest similar results for both annual and perennial plants.

Delayed senescence is also beneficial for improving rhizome quality of *P. polyphylla*. The secondary metabolite content is the main measure of quality of this medicinal plant. Steroid saponins, including pennogenin and diosgenin, are thought to be the principal bioactive components of *P. polyphylla*.^{16,17} Saponins are mostly synthesized in aerial plant parts and then translocated to rhizomes where they are stored. We identified five saponins in *P. polyphylla* and found that the saponin content increased after GA₃ treatment (Fig. 2). Saponin levels increased in all three parts of the rhizome, and particularly in APs (8.5%). Saponins I and II are diosgenin saponins, and the other three are pennogenin saponins. All types of saponin in the rhizome increased, apart from pennogenin saponins in the AB (Fig. 2A). The increased saponin accumulation in rhizomes may be explained by the longer growing phase and increased environmental stress. The minimum temperature during the experiment decreased gradually and dropped below 0°C during the night and early morning hours in the middle and later stages of the study;¹¹ therefore, the plants suffered from additional environmental stress due to the extended growth phase. Plant secondary

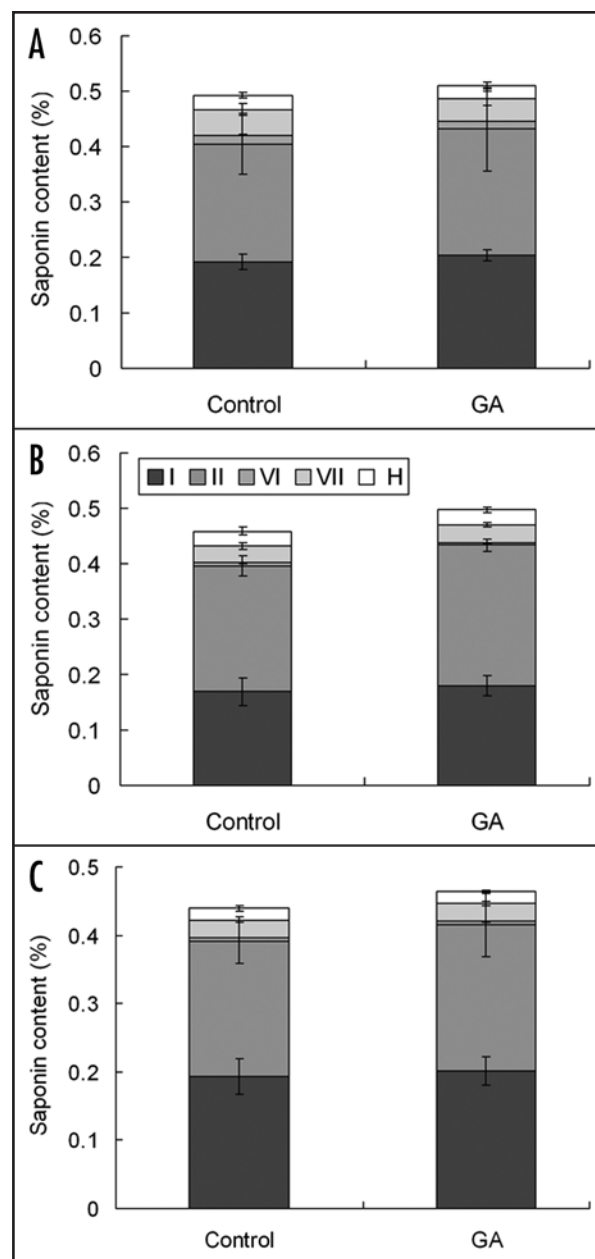


Figure 2. Saponin content of apical buds (A), annual parts (B) and other parts (C) of rhizomes of *P. polyphylla* in 100 mg L⁻¹ GA₃-treated and control plants. I, II, VI, VII and H represents saponins I, II, VI, VII and H, respectively. The rhizomes of the GA₃-treated and control plants (30 plants per treatment) were excavated when the aerial parts of the plants completely senesced. AB, AP and OP were isolated and used to measure saponin content. Values are means \pm SE, n = 3.

metabolites were thought to be simply functionless or metabolic wastes, but more recently, many secondary metabolites have been suggested to have important functions.¹⁸ The activities of enzymes involved in saponin synthesis might be higher in GA₃-treated plants than in controls during the senescence process. Abiotic stress improves the yield of plant secondary metabolites, such as saponins.¹⁹ The major functions of plant secondary metabolites are to enable the survival of biotic and abiotic stresses.¹⁹ Thus,

saponins might act as signal molecules inducing stress signaling pathways to increase tolerance to environmental stress, thereby helping *P. polyphylla* to survive adverse climate conditions.

In summary, the results presented here suggest that rhizome yield and quality of *P. polyphylla* were improved by GA₃-induced retardation of leaf senescence. More detailed studies are needed to characterize the remobilization and recycling of nutrients, such as nitrogen, as well as how the processes are regulated by GA₃ during the senescence process.

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